(For Laboratory Use Only)
Accuratus Lab Services Project # A 2 8 8 8 0 KJP 12-2-19
Test Substance Tracking # TS070119.INT01

PROTOCOL

Standard Operating Procedure for Measuring Antimicrobial Efficacy in Secondary Potable Water Treatment

Test Organism(s):

Legionella pneumophila (ATCC 33152)

PROTOCOL NUMBER

INT01052019.CUST

SPONSOR

International Dioxide Inc.
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North Kingstown, RI 02852

SPONSOR REPRESENTATIVE

Lewis & Harrison, LLC
2461 South Clark Street, Suite 710
Arlington, VA 22202

PERFORMING LABORATORY

Accuratus Lab Services
1285 Corporate Center Drive, Suite 110
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DATE

May 20, 2019

EXACT COPY
INITIALS MS DATE 3-6-2020

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Standard Operating Procedure for Measuring Antimicrobial Efficacy in Secondary Potable Water Treatment

PURPOSE

The purpose of this assay is to evaluate the efficacy of secondary potable water treatment chemical methods against bacteria. This method is in compliance with the requirements of and may be submitted to the U.S. Environmental Protection Agency (EPA).

TEST SUBSTANCE CHARACTERIZATION

According to 40 CFR, Part 160, Subpart F [160.105] test substance characterization as to identity, strength, purity, solubility and composition, as applicable, shall be documented before its use in this study. The stability of the test substance shall be determined prior to or concurrently with this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to Accuratus Lab Services. Accuratus Lab Services will append Sponsor-provided Certificates of Analysis (C of A) to this study report, if requested and supplied. Characterization and stability studies not performed following GLP regulations will be noted in the Good Laboratory Practice compliance statement.

SCHEDULING AND DISCLAIMER OF WARRANTY

Experimental start dates are generally scheduled on a first-come/first-serve basis once Accuratus Lab Services receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is June 5, 2019. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of July 3, 2019. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at Accuratus Lab Services.

If a test must be repeated, or a portion of it, due to failure by Accuratus Lab Services to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing. If the Sponsor requests a repeat test, they will be charged for an additional test. Neither the name of Accuratus Lab Services nor any of its employees are to be used in advertising or other promotion without written consent from Accuratus Lab Services. The Sponsor is responsible for any rejection of the final report by the regulating agencies concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the Accuratus Lab Services final report and notify Accuratus Lab Services of any perceived deficiencies in these areas before submission of the report to the regulatory agency. Accuratus Lab Services will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

Regulatory agencies require that a specific claim be supported by appropriate scientific data demonstrating the efficacy of the product against the claimed test organism. This is accomplished by treating the target organism with the test substance under conditions which simulate as closely as possible, in the laboratory, the actual conditions under which the sanitizer is designed to be used. The experimental design in this protocol meets these requirements. The test system to be used in this study will follow the EPA approved protocol "ECO01040516.CUST". A consistent subculture agar medium will be used to recover survivors in the test, population control and neutralization confirmation control to eliminate recovery variability that may be associated with multiple agar media.

TEST PRINCIPLE

A suspension of test organism cells is exposed to the test substance for a specified exposure time. After exposure, an aliquot of the exposed suspension is transferred to vessels containing neutralizer and assayed for survivors. Appropriate numbers, culture purity, sterility, and neutralization confirmation controls are performed.

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TEST METHOD

Table 1:

Test Organism	ATCC #	Growth Medium	Incubation Parameters
Legionella pneumophila	33152	BCYE agar	35-37°C, **in CO_2**

The test organism to be used in this study was obtained from the American Type Culture Collection (ATCC), Manassas, VA.

Suggested Subculture Agar/Plating Method:
Buffered Charcoal Yeast Extract (BCYE) Agar / Spread-plating

Preparation of Potable Water as the Test Water
Standard tap water from a city water faucet will be autoclave sterilized at 121°C to deplete any chlorine present in the sample. Allow to cool at room temperature minimally overnight. Equilibrate test water to 20±2°C for ≥10 minutes prior to use in testing.

Preparation of the Biocide as the Test Substance
The test substance(s) to be assayed will be used as directed by the Sponsor (See protocol attachment). Transfer 99 mL of test substance to sterile 250–300 mL Erlenmeyer flasks. Place the flasks into a water bath at 20±2°C and equilibrate for ≥10 minutes. Test and control flasks will be tested in duplicate.

Preparation of Test Organism
For *Legionella pneumophila*, the test organism will be streaked to BCYE agar plates from a stock culture. The inoculated agar medium will be incubated for 2-3 days at 35-37°C in CO_2.

Following incubation, add 3-5 mL of sterile deionized water to each plate. Using a sterile loop or other device, gently dislodge the growth from the agar surface. Combine the cultures from all plates and mix thoroughly. The suspension should appear slightly greater than a 4 McFarland turbidity standard to target a minimum of 1 x 10^9 CFU/mL.

Exposure Conditions
The flask containing the test substance will be whirled stopping just before the suspension is added, creating enough residual motion of liquid to prevent pooling of the suspension at the point of contact with test substance. A 1.0 mL aliquot of culture will be added midway between the center and edge of the surface with the tip of the pipette slightly immersed in the test solution. Touching the neck or side of the flasks will be avoided. Swirl the flask to thoroughly mix the contents. Allow the solution to expose for the exposure times.

Test System Recovery
Following each exposure time, 1.0 mL of the inoculated test substance will be transferred to 9 mL of neutralizer. The neutralized material will be vortex mixed. The neutralized contents correspond to the 10^{-1} dilution. Prepare serial dilutions and plate 1.0 mL of 10^{-1} and 0.1 mL of 10^{-1} to 10^{-4} in duplicate.

After each exposure time, an aliquot of the inoculated test substance will be removed from the flask and tested for free residual oxidant.

Incubation and Observation
All subculture plates are incubated for 4-6 days at 35-37°C, in CO_2. Additional incubation may be followed if colonial growth is difficult to visually detect. Subcultures may be stored at 2-8°C for up to 3 days prior to reading. Following incubation (or incubation and storage), the subculture plates will be visually examined for growth.

Representative subculture plates showing growth will be biochemically assayed to confirm or rule out the presence of the test organism. Additional subculturing may be performed, if necessary.

STUDY CONTROLS

Purity Control
A "streak plate for isolation" will be performed on the organism culture and following incubation examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Neutralizer Sterility Control
Prior to or concurrent with testing, the neutralizer used in testing will be evaluated for sterility. A representative sample of neutralizer (1.0 mL), per lot of neutralizer used in testing, will be plated onto the subculture agar medium as in the test. The plate will be incubated and visually examined. The acceptance criterion for this study control is a lack of growth.

Test Substance Diluent Sterility Control
Prior to or concurrent with testing, the test substance diluent used in testing will be evaluated for sterility, if applicable. A representative sample of test substance diluent (1.0 mL), per lot used in testing, will be plated onto the subculture agar medium as in the test. The plate will be incubated and visually examined. The acceptance criterion for this study control is a lack of growth.

Sterile Deionized Water Sterility Control
Prior to or concurrent with testing, the sterile deionized water used in testing will be evaluated for sterility. A representative sample of (1.0 mL), per lot used in testing, will be plated onto the subculture agar medium as in the test. The plate will be incubated and visually examined. The acceptance criterion for this study control is a lack of growth.

Test Substance Sterility Control
A representative sample of prepared test substance (1.0 mL), per sample or lot used in testing, will be plated onto the subculture agar medium as in the test. The plate will be incubated and visually examined. This control is for informational purposes and therefore has no acceptance criterion.

Numbers Control
Transfer 99 mL of prepared tap water to duplicate sterile 250-300 mL Erlenmeyer flasks. Equilibrate the flasks in a water bath to 20±2°C for ≥10 minutes.

Whirl the flask and add 1.0 mL of culture as in the test procedure. Swirl the flask to thoroughly mix the contents.

Following each exposure time, transfer 1.0 mL of the contents to 9 mL of neutralizer as in the test. **The neutralized contents correspond to the 10^{-1} dilution.** Prepare ten-fold serial dilutions to 10^{-6}. Plate 0.1 mL aliquots of the 10^{-4} to 10^{-6} dilutions on BCYE agar and incubate.

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Neutralization Confirmation Control
The following neutralization confirmation control will be performed prior to testing or concurrent with testing. Only the most concentrated test substance dilution needs to be evaluated. Serially dilute the prepared test culture to target $1x10^4 - 1x10^5$ CFU/mL (to target a result of 10-100 CFU plated in each control run). Multiple organism dilutions may be prepared. *(Typically the 10^{-4} and 10^{-5} dilutions will provide a culture in range. Alternate dilutions may be used where appropriate.)* If all the organism dilution(s) used in this control fail to provide adequate numbers which coincides in a failure to meet the acceptance criterion for this study control, the control may be repeated in its entirety.

A. Neutralization Confirmation Control Treatment (NCT)
Add 1.0 mL of test substance to 9 mL of neutralizer and vortex mix. Within approximately 30 seconds, add 0.1 mL of diluted test organism to the neutralized contents and vortex mix. Hold the mixture for a minimum of 2 minutes and spread plate duplicate 1.0 mL and 0.1 mL aliquots. The acceptance criterion for this study control is growth within 1 $\log_{10}$ of the test culture titer (TCT).

B. Neutralizer Toxicity Treatment (NTT)
Add 0.1 mL of diluted test organism to 10 mL of neutralizer and vortex mix. Hold the mixture for a minimum of 2 minutes and spread plate duplicate 1.0 mL and 0.1 mL aliquots. The acceptance criterion for this study control is growth within 1 $\log_{10}$ of the test culture titer (TCT).

C. Test Culture Titer (TCT)
Add 0.1 mL of diluted test organism to 10 mL of sterile deionized water and vortex mix. Hold the mixture for a minimum of 2 minutes and spread plate duplicate 1.0 mL and 0.1 mL aliquots. The acceptance criterion for this study control is growth.

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM
Accuratus Lab Services maintains Standard Operating Procedures (SOPs) relative to efficacy testing studies. Efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including test organism strains for purposes of identification, receipt and use of chemical reagents. These procedures are designed to document each step of efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each efficacy test is assigned a unique Project Number when the protocol for the study is initiated by the Study Director. This number is used for identification of the test subcultures, etc. during the course of the test. Test subcultures are also labeled with reference to the test organism, experimental start date, and test product. Microscopic and/or macroscopic evaluations of positive subcultures are performed in order to confirm the identity of the test organism. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: NA

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
For *Legionella pneumophila* reduction, efficacy performance requirements are a minimum 5 $\log_{10}$ reduction of the test organism within 30 minutes as compared to the numbers control.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol.

If any portion of the protocol is executed incorrectly warranting repeat testing, the test may be repeated under the current protocol number. If the population control fails to meet the minimum requirement or if the neutralization control acceptance criteria is not met and the study fails to meet the efficacy requirements, repeat testing is not required.

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REPORT

The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the organism strains used, description of media and reagents, description of the methods employed, tabulated results and conclusion as it relates to the purpose of the test, and all other items required by 40 CFR Part 160.185.

PROTOCOL CHANGES

If it becomes necessary to make changes in the approved protocol, the revision and reasons for changes will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

Standard operating procedures used in this study will be the correct effective revision at the time of the work. Any minor changes to SOPs (for this study) or methods used will be documented in the raw data and approved by the Study Director.

TEST SUBSTANCE RETENTION

It is the responsibility of the Sponsor to retain a sample of the test substance. All unused test substance will be discarded following study completion unless otherwise indicated by Sponsor.

RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at Accuratus Lab Services for a minimum of five years for GLP studies or a minimum of six months for all other studies following the study completion date. After this time, the Sponsor (or the Sponsor Representative, if applicable) will be contacted to determine the final disposition. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of final study report.
7. Study-specific SOP deviations made during the study.

Facility Specific Documents

The following records shall also be archived at Accuratus Lab Services. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

REFERENCES

1. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method. In Official Methods of Analysis of the AOAC, 2013 Edition.
2. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Uses of Antimicrobial Agents, September 4, 2012.
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2300: Sanitizers for Use on Hard Surfaces- Efficacy Data Recommendations, September 4, 2012.
4. ASTM E645-13; Standard Practice for Evaluation of Microbicides Used in Cooling Water Systems.

DATA ANALYSIS

Calculations

Determine the CFU/mL for the test sample and numbers control using counts of 0-300.

$$\text{CFU/mL} = \frac{(\text{average CFU for } 10^{-x}) + (\text{average CFU for } 10^{-y})}{(10^{-x} + 10^{-y}) \times (\text{volume plated})}$$

where 10^{-x} and 10^{-y} are the dilutions plated. Where no survivors are found in all test dilutions, the final value will be expressed as <1 CFU/mL. If all dilutions are TNTC, substitute >300 at the highest (most dilute) dilution.

The geometric mean value for the population control will be determined and used to calculate percent reduction if multiple time points are evaluated in the control. The geometric mean value of the test results will be determined and used to calculate percent reduction if more than one replicate is performed.

$$\text{Mean } \text{Log}_{10} \text{ Density} = \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2}{N}$$

where: X equals CFU/mL
N equals number of test replicates or population control time points

Log_{10} Reduction = Log_{10} (CFU/mL in the numbers control) – Log (CFU/mL in the test sample)

Log_{10} Difference in the Neutralization Confirmation Control = Log_{10} (Average CFU in TCT) – Log_{10} (Average CFU in NCT or NTT)
An appropriate dilution and volume plated will be used to determine the log_{10} difference in the neutralization confirmation control.

Statistical Analysis:
None used

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STUDY INFORMATION

(All blank sections are verified by the Sponsor or Sponsor Representative as linked to their signature, unless otherwise noted.)

Test Substance (Name & Batch Numbers) exactly as it should appear on final report:

Test Substance Name	Lot/Batch Number
Adox (TM) BCD-7.5	1803222AHE
	1902181AHA
	1903071AHA

Testing at the lower certified limit (LCL) is required for registration, no aged batch is necessary.

Product Description:

❑ Quaternary ammonia ❑ Peracetic acid ❑ Iodophor
❑ Peroxide ❑ Sodium hypochlorite ☑ Other chlorine dioxide

Approximate Test Substance Active Concentration (upon submission to Accuratus Lab Services):

7.5% Sodium chlorite

(This value is used for neutralization planning only. This value is not intended to represent characterization values.)

Neutralization/Subculture Broth: ❑ _____

(NOTE: All broth must also serve as an appropriate growth medium for the test organism)

☑ Accuratus Lab Services' Discretion. By checking, the Sponsor authorizes Accuratus Lab Services, at their discretion, to perform neutralization confirmation assays at the Sponsor's expense prior to testing to determine the most appropriate neutralizer. (See Fee Schedule).

Storage Conditions

☑ Room Temperature
❑ 2-8°C
❑ Other _____

Hazards

❑ None known: Use Standard Precautions
☑ Material Safety Data Sheet, Attached for each product
❑ As Follows: _____

Product Preparation

❑ No dilution required, Use as received (RTU)
☑ *Dilution(s) to be tested:

_____ defined as _____ + _____
(example: 1 oz/gallon) (amount of test substance) (amount of diluent)

❑ Deionized Water (Filter or Autoclave Sterilized)
❑ Tap Water (Filter or Autoclave Sterilized) - All tap water is softened; the water hardness for the batch of tap water used will be determined and reported.
❑ AOAC Synthetic Hard Water: _____PPM
☑ Other See Sponsor provided chlorine dioxide generation method

Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.

Test Organism(s): ☑ *Legionella pneumophila* (ATCC 33152)

Exposure Time: 5 minutes, 15 minutes, 30 minutes

Exposure Temperature: ❑ 25 ± 1°C*
☑ Other: 20±2°C

Organic Soil Load:

❑ Minimum 5% Organic Soil Load (Fetal Bovine Serum)
☑ No Organic Soil Load Required
❑ Other: _____

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TEST SUBSTANCE SHIPMENT STATUS
(This section is for informational purposes only.)
☑ Test Substance is already present at Accuratus Lab Services.
☐ Test Substance has been or will be shipped to Accuratus Lab Services.
Date of expected receipt at Accuratus Lab Services:_____
☐ Test Substance to be hand-delivered (must arrive by noon at least one day prior to testing or other arrangements made with the Study director).

COMPLIANCE
Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) in accordance to standard operating procedures.
☑ Yes
☐ No (Non-GLP or Development Study)

REGULATORY AGENCY(S) THAT MAY REVIEW DATA
☑ U.S. EPA
☐ Health Canada
☐ Therapeutic Goods Administration (Australian TGA)

PROTOCOL MODIFICATIONS
☐ Approved without modification
☑ Approved with modification
See attachment for chlorine dioxide stock solution preparation and Lovibond MD100 colorimeter use.
Dilute each prepared stock solution in prepared potable water to target a starting dose of ~0.8 ppm ClO2 for testing (account for the demand of the tap water).
Measure the chlorine dioxide once after each timepoint (per test flask). Additional chlorine dioxide measurements may be performed as need.
For the neutralization confirmation control, prepare the test substance as in the test, titrations are not needed for this control.

PROTOCOL ATTACHMENTS
Supplemental Information Form Attached - ☑ Yes ☐ No

TESTING FACILITY MANAGEMENT VERIFICATION OF 40 CFR PART 160 SUBPART B (160.31(D))

Identity, strength, purity, and uniformity, as applicable, of the test lots has been or will be completed prior to efficacy testing: ☑ **Yes** ☐ **No*** ☐ **Not required, Non-GLP testing requested**

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☑ **Yes** ☐ **No***

Optional Information to complete as applicable:
☐ A Certificate of Analysis (C of A) may be provided for each lot of test substance. If provided, the C of A will be appended to the report.
☑ Testing has been or will be conducted under protocol or study #:
INT01051719.VTT

Stability testing of the formulation has been or will be completed prior to or concurrent with efficacy testing:
☑ **Yes** ☐ **No*** ☐ **Not required, Non-GLP testing requested**

If yes, testing was or will be performed following 40 CFR Part 160 GLP regulations: ☑ **Yes** ☐ **No***

Optional Information to complete as applicable:
☐ Testing has been or will be conducted under protocol or study #:
_____

**If testing information is not provided or is not performed following GLP regulations, this will be indicated in the GLP compliance statement of the final report.*

PROPRIETARY INFORMATION

THIS DOCUMENT IS THE PROPERTY OF AND CONTAINS PROPRIETARY INFORMATION OF ACCURATUS LAB SERVICES. NEITHER THIS DOCUMENT, NOR INFORMATION CONTAINED HEREIN IS TO BE REPRODUCED OR DISCLOSED TO OTHERS, IN WHOLE OR IN PART, NOR USED FOR ANY PURPOSE OTHER THAN THE PERFORMANCE OF THIS WORK ON BEHALF OF THE SPONSOR, WITHOUT PRIOR WRITTEN PERMISSION OF ACCURATUS LAB SERVICES.

APPROVAL SIGNATURES

SPONSOR:

NAME: Ms. Christina Swick TITLE: Agent

SIGNATURE: Christina M. Swick DATE: November 19, 2019

PHONE: (202) 393 - 3903 EMAIL: cswick@lewisharrison.com

For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.

Other individuals authorized to receive information regarding this study: ☐ See Attached

Accuratus Lab Services:

NAME: Mathew Sathe
Study Director

SIGNATURE: Mathew Sathe DATE: 12-2-19
Study Director

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Chloride Dioxide Stock Solution Preparation (prepares ~60 ppm stock in tap water)

Pipette 0.2 mL of ADOX BCD-7.5 (~7.5% sodium chlorite) into an appropriately sized bottle, per lot.
Pipette 5 mL of 10% HCl solution into the bottle and gently swirl for a few seconds.
Start the stop watch and cover with parafilm.
After 2 minutes, remove the parafilm from the bottle and carefully pour 95 mL of prepared sterile tap water into the bottle.
Cap the bottle and slowly invert 3-4 times to mix.
Store bottle in the refrigerator until ready to use.
Tap water demand is ~150 µL stock solution per 200 mL tap water.
Analyze the stock concentration following the Lovibond MD100 instructions below.
Re-analyze the stock concentration if not used within an hour from the previous analysis.

Chlorine Dioxide Measured by Lovibond MD100 Colorimeter

Lovibond MD100 Colorimeter Setup**:** Turn the unit on by pressing the [ON/OFF] key. Select [MODE] until the arrow on the screen is above Tablet.

Zeroing the Lovibond MD100

Fill a vial with DI water up to the 10 mL mark and screw the cap on.
Place the vial in the sample chamber, making sure the arrow marks are aligned.
Press the [ZERO/TEST] key.
The "Method" symbol flashes for approximately 8 seconds and the zero calibration is complete.
Note: The Lovibond MD100 Colorimeter may shut off after inactivity. Re-zero the unit each time the colorimeter is turned back on.

Sample Testing (for samples of approximately 0.02 to 11 mg/L ClO_2):

Add one DPD No. 1 tablet straight from the foil (avoid touching) into Vial 1 and crush the tablet using a clean stirring rod (Vial 1).
Fill a second vial with 10mL of sample (Vial 2).
Add one Glycine tablet straight from the foil (avoid touching) to Vial 2 containing the sample and crush the tablet using a clean stirring rod.
Close Vial 2 tightly with the cap and swirl gently several times until the tablet is dissolved.
Transfer the contents of Vial 2 into Vial 1 with the DPD tablet.
Close the vial tightly with the cap and swirl gently several times until the tablet is dissolved.
Place the vial in the sample chamber, making sure that the arrow marks are aligned.
Press the [ZERO/TEST] key. The method symbol flashes for approximately 3 seconds.
The result is shown in the display in mg/L ClO_2.

NOTES: Avoid loss of chlorine dioxide by pipetting and shaking.

The analysis must immediately take place after taking the sample.

Concentrations above 19 mg/L chlorine dioxide can lead to results showing 0 mg/L. In this case, the sample must be diluted with deionized water into the range of the instrument and a dilution factor will be used to calculate the results of the solution. See calculation section.

Other error codes may present on the screen, consult the instruction manual for the error code reason and troubleshoot the error.

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CALCULATIONS

1mg/L = 1ppm

Dilution Factor (D):

$$Dilution\ Factor\ (D) = \frac{(\text{Total Volume, mL})}{(\text{Volume of Sample Aliquot, mL})}$$

Sample Calculation if Dilution was performed:

$$ClO_2(\frac{mg}{L}) = (Reading\ from\ Lovidbond\ Instrument)\ x\ D$$

Preparation of 0.8ppm Chlorine dioxide solution

a. Add approximately 900 mL of tap water to a 1 L volumetric flask.
b. Determine the amount of 80 ppm chlorine dioxide to add to the 1 L volumetric flask by the following equation:

$$V = \frac{(0.8\ ppm\ ClO_2\ Target)\ x\ (1000\frac{mL}{L})}{(Actual\ ppm\ of\ ClO_2\ stock\ solution)} + (Determined\ ClO_2\ Demand\ volume\ in\ mL\ x\ 5)$$

Where V = Volume in (mL) of ClO_2 required to achieve 0.8 ppm and provide demand for 1L
5 = a factor to convert the determined chlorine demand from 200 mL to 1000 mL

c. Determine the concentration of chlorine dioxide by following the Chlorine Dioxide by Lovibond MD100 Colorimeter method to determine the concentration of the solution.

REFERENCE:

Lovibond MD100 Colorimeter Chlorine Dioxide Instruction Manual. Version SC450_V1_12/2010.